REPRODUCTION AND EARLY DEVELOPMENT OF THE SPIONID POLYCHAETE, SCOLECOLEPIDES VIRIDIS (VERRILL)

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As far as is known the sedentary polychaete, *Scolecolepides viridis* (Verrill), is confined to the east coast of North America where it is found from Newfoundland to South Carolina (Pettibone, personal communication). The worm is most common intertidally but occurs down to 20 fathoms. It burrows in a variety of substrata and is particularly abundant in sand (Verrill, 1873; Webster, 1879; Webster and Benedict, 1884; Wells and Gray, 1964). The worm is most frequently found in areas of reduced salinity (Cowles, 1931; Stickney, 1959; Smith, 1964). The adults inhabit vertical mucus-lined burrows and feed on the surface deposit aided by a pair of ciliated tentacles that draw food into the region of the mouth where it is engulfed by eversion of the pharynx. Sanders *et al.* (1962) found the gut contents of specimens from Barnstable Harbor, Massachusetts, to consist of sand, detritus, diatoms, filamentous algae, and a nematode.

Little information is available regarding the reproduction and early development. Dr. David Dean (personal communciation) found specimens with eggs and sperm in February and March in the Mystic Estuary, Connecticut, and Dr. Marian Pettibone (personal communication) noted worms massed with eggs in the Oyster River, New Hampshire, at the beginning of April. Dr. J. P. Moore (unpublished manuscript) reported that breeding occurred in the Woods Hole region in July and August. Mead (1879) followed the first few divisions of an egg of a worm which he called *Scolecolepis viridis* but which, in my opinion, was wrongly attributed to this species.

Materials and Methods

The population chosen for the study is located in intertidal mud-sand at Lawrencetown, Nova Scotia. The worms are living in estuarine conditions, often experiencing considerable changes in salinity during a tidal cycle (1–32‰). The worms are not distributed evenly over the flat but show marked aggregation (maximum density of adults—1000 per m.^{2}) which cannot be correlated with particle size of the substratum or with its organic content.

As the sole description of the species (Verrill, 1873) is brief and unillustrated it is necessary to include at this point a redescription of the worm before considering its early development. The adult worm is composed of up to 250 segments and has an approximate length of 140 mm. and a width of 3 mm. It may be colored various shades of green or brown but invariably has red branchiae.

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The body, which in cross-section is convex dorsally and flattened ventrally, is widest at the anterior end and tapers posteriorly. The prostomium, whose anterior end forms distinct frontal horns, narrows posteriorly and usually bears two pairs of eyes (often only one pair or none) (Fig. 1A). The grooved tentacular palps arising from the peristomium are short and stout and spotted with black pigment. A ciliated vestibule bounded laterally by thick lips leads to the gut (Fig. 1B). The bilobed parapodia begin on the first post-peristomial segment. All parapodia bear short capillary setae (Fig. 1E) with spreading bundles of long setae at the dorsal and ventral extremities (Fig. 1C, D). The hooded

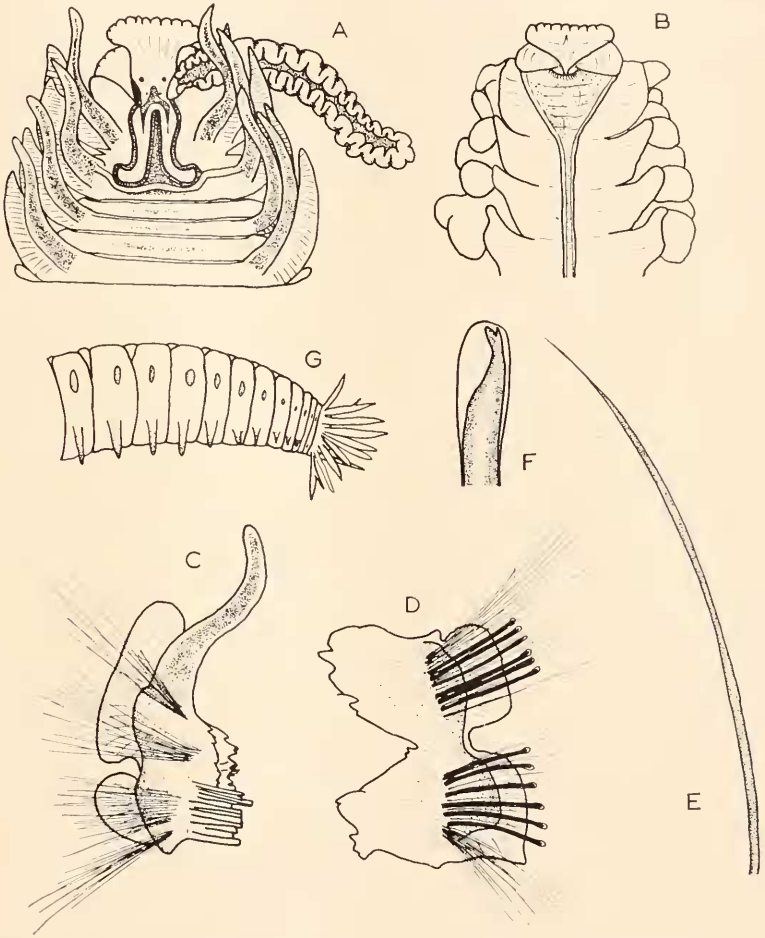


FIGURE 1. External morphology of *Scolecolepides viridis*. A, Dorsal view of the anterior end × 40; B, ventral view of the anterior end × 40; C, right anterior parapodium × 30; D, left posterior parapodium × 30; E, capillary seta × 130; F, bidentate tip of crotchet from posterior end × 250; G, lateral view of the posterior end × 40. In A, B and G, setae have been omitted for clarity.

bidentate crotchets (Fig. 1F) are absent from the anterior setigers, first appearing in the neuropodium between segments 20–50 and in the notopodium between segments 35–65. There are up to 11 crotchets per lobe. Immediately behind the bristle bundles on each segment are neuropodial and notopodial post-setal lamellae, which become less prominent more posteriorly. The ligulate branchiae, of which there is one pair per segment, begin on the first setiger. They are prominent in the anterior third of the body where they are fused to the post-setal lamellae. The branchiae become progressively smaller towards the middle of the body where they eventually disappear. The posterior end is truncate and the terminal anus is surrounded by cirri (8–20) which are sometimes dichotomously branched (Fig. 1G). The worm has ciliated bands intersegmentally and also a band in the middle of each segment which continues laterally on to the parapodia. On the dorsum of the first two segments there is a pair of ciliated epaulettes (Fig. 1A). The ciliation of the dorsum and branchiae is stronger than that of the rest of the body. These dorsal cilia are the main propulsive force behind the water currents aerating the burrow of the worm. Water is swept towards the posterior end along the tunnel formed by the arched branchiae. Over the rest of the body surface the cilia beat towards the anterior end. Occasionally individuals reverse the direction of the ciliary beat.

To obtain evidence for the onset of breeding, worms were collected at monthly intervals and examined for the presence of sexual products in the coelom. As the breeding season neared, weekly collections were made. On the high tide preceding or following the collection of the adults, three 500-liter pump samples were taken from the sea water overlying the flat. The water was filtered through 125-μ and 63-μ mesh nets, and examined for eggs and young larval stages of the worm.

Permanently installed maximum-minimum thermometers were used to record the air, mud, and sea water temperatures. Where possible a portable induction salinometer was used to determine the salinity, otherwise water samples were processed in the laboratory, using the Mohr method.

Adult worms were maintained in the laboratory in flat-bottomed glass culture dishes containing filtered sea water from the sampling site. The aerated sea water in the vessels was changed twice a week and normally kept at a temperature approximating that prevailing in the field. The worms were supplied with lengths of glass tubing which they used as "burrows." The majority of larval experimental cultures resulted from naturally spawned eggs and sperm, but occasionally eggs were fertilized using sperm cut from mature males. After fertilization eggs were transferred to clean sea water. Some were allowed to develop at 2°, 10° and 20° C. in 30‰ sea water, whilst others were kept at temperatures of 2° C. or 10° C. and allowed to develop at different salinities (5, 10, 15, 20 and 30‰).

Both adults and larvae were fed on a sea-water suspension of *Ulva* which had been dried and then finely powdered. Living developmental stages were photographed in watchglasses, the ciliated larvae being slowed when necessary by addition of $MgCl_2$ crystals to the sea water. The photographs were taken by transmitted light through a Leitz Orthrolux photomicrographic unit, using Panatomic X film and a red filter over the light source to increase the contrast.

Breeding Period

Seasonal variation in the percentage of worms containing genital products

Ripe males and gravid females were present in approximately equal numbers (Fig. 2). When sampling started in late February, 1963, 96% of the population contained gametes. During March this figure dropped to 11% and by the end of April only a few ripe males remained. In June, July, August and September no gamete-bearing animals were found. Gravid females appeared in October and by the latter part of November ripe males were also present, bringing the

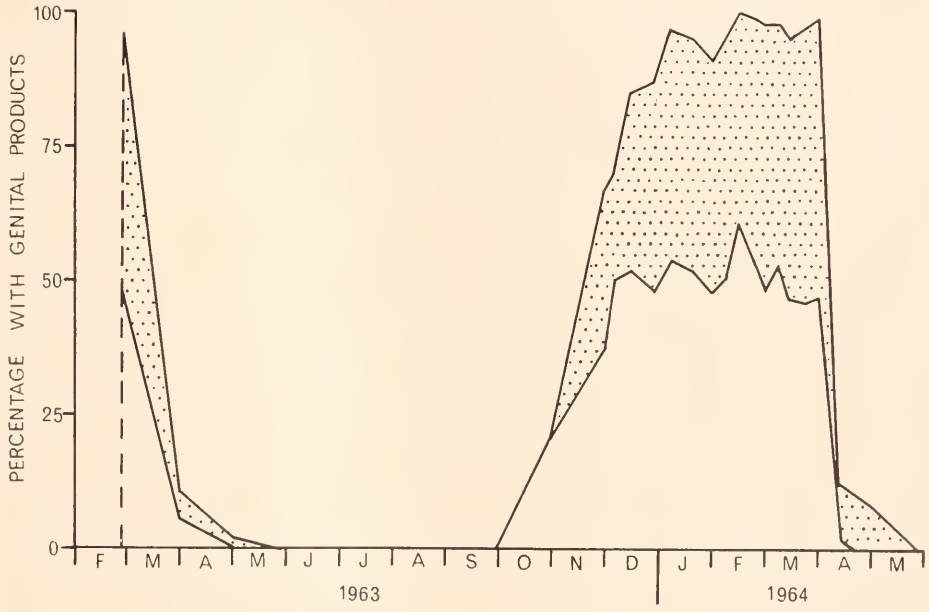


Figure 2. Seasonal variation in the percentage of worms containing genital products. Open area = females; stippled = males.

total percentage of worms with genital products to 70%. The percentage continued to rise and by the beginning of January, 1964, did not differ significantly from 100%. During January, February and March the percentage stayed at this high level, but the first week in April saw a marked decline to 12%. Thereafter, as in the previous year, only a few ripe males remained until the end of May. Sharp declines in the percentage of worms with genital products suggest that maximum breeding activity occurred during March in 1963 and at the end of March and beginning of April in 1964.

The data also reveal that the worms must reach maturity in less than one year, since all worms are gravid at one time.

Seasonal variation in the quantity of genital products in adult worms

Four categories, similar to those used by Joyner (1962), were adopted to record the quantity of gametes present in the reproductive region of the worms (see legend to Fig. 3).

In late February, 1963, the majority of the males were half-full with gametes and at least 25% had coeloms that were full (Fig. 3). A high percentage of the females (44%) were full. By the end of March all the females were spent individuals as were the majority of the males. It was not until the following October that gametes again appeared, and individuals collected in November and December were predominately those with gametes present only in the base of the parapodia. By the end of January, 1964, however, many were half-full with gametes. In February half-full worms predominated, as did full worms by the end of the following month, although several spent worms of both sexes were present by this time. Only spent animals could be found by the end of April.

The lack of full animals before February would indicate that breeding was not possible prior to this. In addition the Figure shows that all reproduction must have ceased by the end of March in 1963 and by the end of April in 1964. Fig-

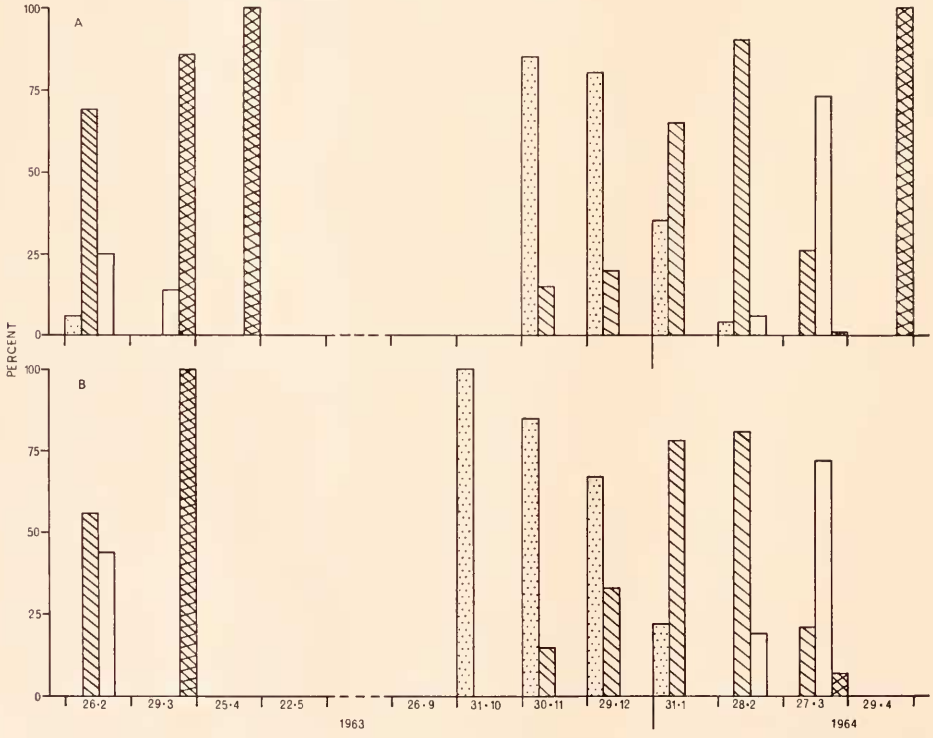


FIGURE 3. Seasonal variation in the quantity of genital products in the coelom of (A) males, and (B) females. Numbers in each category are expressed as a percentage of the total sample. Stippled bar = gametes in the base of the parapodia; bar with diagonal lines = coelom half-full; open bar = coelom full; cross-hatched bar = spent worms.

TABLE 1

Number of eggs and larvae per m.³ in the sea water overlying the sampling site. Data are means from three taken on each date.

Stage of development	1963					1964									
	26.2	29.3	25.4	23.5	26.6	28.2	14.3	21.3	27.3	7.4	14.4	21.4	29.4	28.5	27.6
Unfertilized and dead oocytes	—	30	—	—	—	—	—	9	24,000	249	65	32	37	—	—
Early cleavage	—	350	—	—	—	—	—	5	80,750	510	32	6	13	2	—
Late cleavage and trochophore stages	—	20	—	—	—	—	—	—	—	162	2	—	2	—	—
1- to 3-setiger larvae	—	—	40	—	—	—	—	—	—	—	41	32	—	—	—
4- to 7-setiger larvae	—	—	—	—	—	—	—	—	—	—	3	2	12	2	—
8- to 11-setiger larvae	—	—	—	13	—	—	—	—	—	—	—	—	2	5	—

ures 2 and 3 both show that, initially, development of the male gametes lagged behind that of the female. This phenomenon was seemingly repeated at the end of the breeding period when ripe males were still present after all traces of gametes had disappeared in the females.

Presence of eggs and larvae in the plankton

At the end of March, 1963, eggs were found in the plankton, the majority of them being at early cleavage (Table 1). Only 3-setiger larvae were present in late April but all had reached the 8- to 11-setiger stage by the end of May. Plankton samples taken in late June were free of young stages of *S. viridis*. The sea water overlying the sampling site remained clear until the end of March, 1964, when there was a large influx of eggs into the plankton. As at this time in the previous year the majority of the eggs were at early cleavage. By the end of the first week in April trochophores were present in the plankton. Throughout April the larvae increased in number of setigers until by the end of the month the larvaae possessed 4–11 setigers. A sample at the end of May showed few larvae remaining, the largest number being at the 8- to 11-setiger stage.

Although the scant data for 1963 show only that breeding was taking place during March, those for 1964 indicate that a very large spawning burst occurred at the end of March in this year. Numbers of animals obtained in the plankton samples are too low to draw any firm conclusions as to mortality and growth rate in the field, although the figures suggest that the larvae remain almost two months in the plankton, by which time they have reached the 10- to 11-setiger stage.

SPAWNING

The gonads (one pair/segment), which are attached to the nephridia, are absent only from the first 40–50 segments and the last 45–50 segments. During the breeding season the males and females can be readily distinguished by the color of their sexual products which are visible through the body wall. Sperm appear white and the oocytes orange/brown. The sperm are of the usual short-headed polychaete type (Franzén, 1956) with a pointed acrosome (Fig. 4). When mature, coelomic oocytes are disc-shaped with a diameter of 200–260 μ.

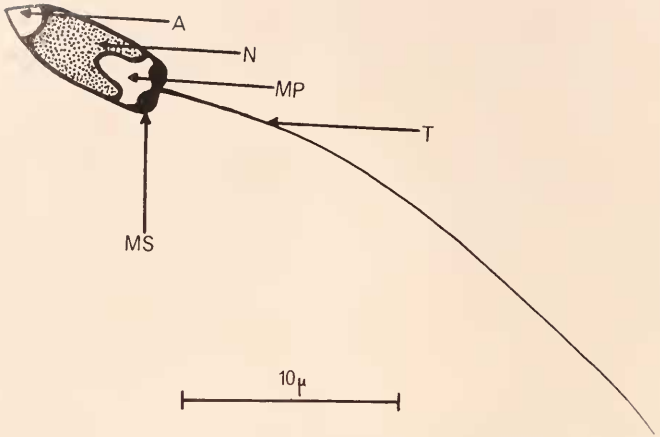


FIGURE 4. Spermatozoön of *S. viridis*. A = acrosome; N = nucleus; MP = middle portion; MS = mitochondrial spheres; T = tail. (Nomenclature according to Franzén, 1956.)

The egg-membrane is thick, the outer surface with a lattice-work of ridges giving it the appearance of a honeycomb, and the inner surface smooth (Fig. 5). Imbedded in the yolk round the periphery of the cell are 10–18 thin-walled vesicles connecting by narrow necks with small pores in the egg membrane. The nucleus and the membrane vesicles are translucent and colorless whilst the opaque yolk has a light green coloration in transmitted light. The number of oocytes produced by each female varies from 10,000 to 16,000.

Eggs and sperm are shed through mixonephridia (Goodrich, 1945) whose external openings are situated dorso-laterally and anterior to the notopodium. Spawning was not observed in the field but worms held in the laboratory shed their gametes whilst in their "glass burrows." The eggs and sperm were wafted out of the burrows in the currents set up by the cilia of the body surface. It is likely, therefore, that spawning in the field takes place in a similar fashion, especially as repeated excursions into the field by day and night failed to reveal any adults lying on the surface of the sand or swarming in the sea water over the sampling site. It must be noted, however, that the adults are good swimmers, moving through the water tail-first with a spiral motion.

Under controlled conditions in the laboratory, sudden salinity changes from 30‰ to 10‰ and from 10‰ to 30‰ led to spawning of 15% of the ripe females and of slightly fewer males (11%). Experiments in which animals were exposed to sudden temperature changes (within minutes) of 5° C. or 10° C. gave inconclusive results. It is clear that neither of the above factors is likely to be a major cause of spawning in the field population of *S. viridis*. No evidence was obtained to suggest that either sex was able to induce spawning in the other as a result of shedding, as is the case in many other invertebrates (Thorson, 1950).

EARLY DEVELOPMENT (At 10° C. and salinity of 30‰)

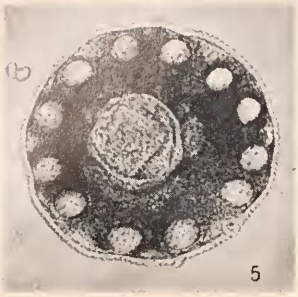
After fertilization, which could not be successfully accomplished in the laboratory below 5‰, the cytoplasm becomes detached from the inner side of the

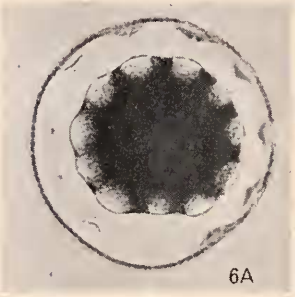
egg-membrane and starts to round off within an inner fertilization-membrane. The membrane-vesicles accompany the migrating cytoplasm and in so doing place their connection to the egg-membrane under tension, causing the egg-membrane to indent in the region of the pores (Fig. 6A). Simultaneously the disc-shaped egg takes on a more spherical form with the vesicles distributed equatorially (Fig. 6B). Following the initial contraction of the plasm, the membrane-vesicles increase in size and migrate from within the cytoplasmic mass, drawing tongues of material with them. The strain on the egg-membrane is thus relieved and the indentations disappear (Fig. 7). The tongues of cytoplasm retract into the central mass (Fig. 8), which rounds off, leaving the membrane-vesicles distributed between it and the egg wall (Fig. 9). This stage is reached in about 12 hours. As cleavage proceeds the vesicles become less visible and eventually disappear. The behavior of the vesicles is consistent with the function of volume-regulation ascribed to them in another spinoid worm by Hannerz (1956).

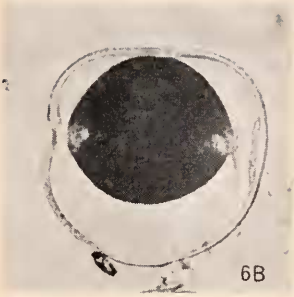
Cleavage is typical of the annelids, being unequal and spiral. The first cleavage produces a CD blastomere which is much larger than the AB blastomere (Fig. 10). At the 4-cell stage the D cell is readily distinguished by its large size (Fig. 11). After formation of the micromeres by a horizontal third cleavage (Fig. 12), division proceeds in a spiral fashion, making it almost impossible to follow in detail (Figs. 13 and 14). Gastrulation by epiboly occurs approximately two days after fertilization. The mass of cells now begins to take on a definite shape with a pointed front end (prostomium) from which apical cilia project through the egg-membrane (Fig. 15). As the prototroch differentiates, more cilia extend through the membrane and the embryo starts to rotate slowly, due to active beating of the cilia. This early larval stage can best be termed a pretrochophore since the ciliation is not yet strong enough to allow the larva to perform free-swimming movements.

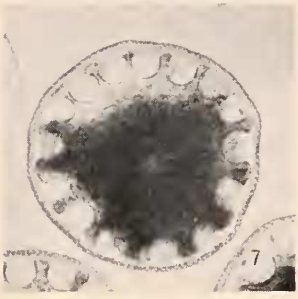
The trochophore commences active swimming 3–4 days after fertilization and, unlike the adult, is strongly photopositive. Whilst swimming the larva slowly rotates about its longitudinal axis. The larva by this time has filled the space that existed between it and the egg-membrane and the membrane becomes incorporated as the larval cuticle. The crenulations that were so marked in the egg wall during cleavage stages are beginning to flatten out (Fig. 16). The prostomium has become rounded and the tuft of apical cilia more prominent. The prototroch girdle, which is formed on the raised area destined to be the peristomium, is composed of clumps of cilia which originate from well-defined pits in the body wall. Mid-dorsally there is a wide gap in the prototrochal ciliation. Ventrally the blastopore is readily visible. The region from which the gut will differentiate is seen as a dense mass of endoderm cells packed with yolk.

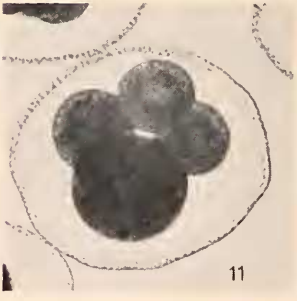
As the larva elongates the prostomium becomes dome-shaped and a pair of brown eyes, set wide apart, appears on it. A telotroch develops in the pygidial region and as in the prototroch the cilia occur in clumps with a mid-dorsal gap. The larval cuticle has by this time lost its sculpturing except at the posterior end. A small mouth leads into the yolk-filled gut in which three regions can be distinguished. Larval bristles on a raised area behind the prototroch mark the position of the first setiger (Fig. 17). The larva at this stage is approximately four days old.

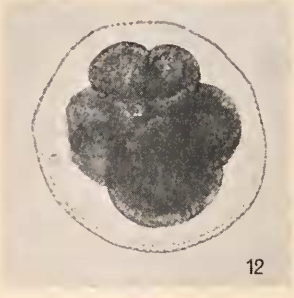


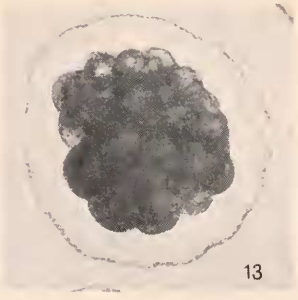












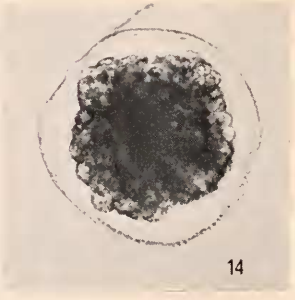


FIGURE 5. Disc-shaped unfertilized egg.

FIGURE 6A. Fertilized egg. The cytoplasm has contracted away from the egg-membrane, drawing the membrane-vesicles with it. 6B. Side view of the same stage, showing the equatorial distribution of the vesicles.

By the time the pale green larva has reached the 3-setiger stage (Fig. 18) the prostomium has become flattened anteriorly and the long apical cilia have disappeared. At least three pairs of "sensory cilia" (*senso* Wilson, 1928) occur on the front edge of the prostomium and several are also found scattered on the underside of the prostomium and ventro-laterally on the pygidium. A second pair of eyes, smaller than the first, appears dorsally. The cilia of the prototroch have developed more strongly in the dorsal region, forming a continuous girdle broken only at the mid-dorsal gap. Ventrally the cilia of the prototroch are indistinguishable from the short cilia which are scattered over the concave ventral surface of the prostomium and peristomium. The cilia extend posteriorly as a neurotroch, ending in an inconspicuous ciliated pit on the second setiger. The slit-like ciliated vestibule is open anteriorly and ventrally and is bounded laterally by thick lips. The three regions of the gut are now fairly distinct. The mid-gut, which opens to the hind-gut through a narrow neck, is characterized by dark pigmentation. The few yolk granules that remain are confined to the mid-gut. Movement of food through the gut is facilitated by ciliation along its whole length and by periodic peristaltic waves starting at the anterior end. The larval setae are longest on the first segment and occur on the following segments in decreasing length. They are concentrated dorso-laterally in the notopodial position of each segment, and are finely serrated on their anterior margin.

The 3-setiger stage is reached in about 10 days but there is then a growth pause and the fourth setiger is not added for another 10 days. The pause may result in part from the final disappearance of the yolk granules and the larva having thus to rely entirely for its food supply on organic material removed from the sea water. It is at this stage that the greatest mortality occurred among larvae in the cultures. When once the larvae had passed this critical stage in their development mortality was much reduced.

As the larva develops beyond the 3-setiger stage the parapodia become more prominent, and a gastrotroch appears on the third segment. A smaller gastrotroch is formed on either side of the ciliated pit. By the time the larva reaches the 5-setiger stage (Fig. 19), it is approximately 425 μ long and 30 days old.

At the 9-setiger stage gastrotrochs are present on segments 3, 5, 7 and 9. Conspicuous nototrochs occur on segments 2, 3, 4 and 5, and smaller dorsal bands of cilia on segments 6, 7, 8 and 9. The notopodial (including branchial) and neuropodial regions of the parapodia have differentiated and small unserrated adult setae are beginning to appear. The tentacular palps are noticeable as small protuberances on the peristomium.

The larvae start to metamorphose at the 9- to 10-setiger stage. They be-

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FIGURE 7. Membrane-vesicles migrating from within the cytoplasmic mass, drawing tongues of material with them.

FIGURE 8. Tongues of cytoplasm retracting into the central mass.

FIGURE 9. Membrane-vesicles distributed between the egg-membrane and the rounded central mass of cytoplasm.

FIGURE 10. Two-cell stage, showing the AB blastomere and the larger CD blastomere.

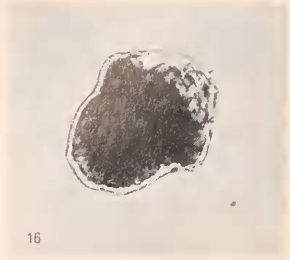
FIGURE 11. Four-cell stage with the large D cell clearly visible.

FIGURE 12. Eight-cell stage, showing formation of the micromeres.

FIGURE 13. Mid-cleavage with macromeres still visible.

FIGURE 14. Late cleavage.

FIGURE 15. Pre-trochophore with apical cilia protruding through the egg-membrane.



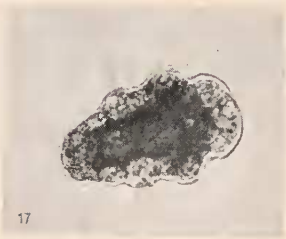


FIGURE 16. Ventral view of a trochophore with the blastopore still visible.
FIGURE 17. Ventral view of 1-setiger larva.
FIGURE 18. Dorsal view of 3-setiger larva.
FIGURE 19. Dorsal view of 5-setiger larva.
FIGURE 20. Side view of 9-setiger larva.
FIGURE 21. Dorsal view of a recently metamorphosed individual with 10 setigers.

come photonegative in their reactions, and continually test the substratum for its suitability for burrowing, apparently using the "sensory cilia" of the prostomium. When no suitable substratum is available the larvae are able to postpone metamorphosis and will reach the 13-setiger stage, and then, in many cases, metamorphose successfully even in clean glass dishes. Many larvae begin to lose their larval bristles before settling but the majority are lost as the worms first burrow in the substratum. As noted by Dean (1965) for *Streblospio benedicti,* metamorphosis accelerates the development of the tentacular palps and branchiae (compare Figure 20 of a 9-setiger larva and Figure 21 of a recently metamorphosed individual). Adult setae also rapidly develop in both notopodium and neuropodium. The prototroch, gastrotrochs, neurotroch, ciliated pit and telotroch disappear during metamorphosis, although the nototrochs and oral cilia persist into adult life. The fully-metamorphosed 10-setiger worm is 750 μ long and approximately 40 days old.

As the young worms grow by addition of more segments, crotchets appear first in the neuropodium and then in the notopodium. Anal cirri become visible round the terminal anus, and the now-prominent branchiae become ciliated. By this time the young worm is readily recognizable as a young *S. viridis* and has adopted a position in a vertical burrow similar to that of the adult.

Effect of temperature on early development (At 30‰)

It can be seen in Figure 22 that sea water temperature affects the rate of growth. In laboratory cultures kept between 2° C. and 20° C. the growth rate increased with temperature until the 3-setiger stage. At 10° C. and 20° C. development continued after a growth pause, and metamorphosis was reached approximately twice as fast at 20° C. as at 10° C. At 2° C., however, growth virtually ceased when the 3-setiger stage was reached and in no cultures did the larvae proceed beyond the 3- to 4-setiger stage. That this is a temperature effect was demonstrated by increasing the temperature of the sea water in the cultures halted at the 3-setiger stage from 2° C. to 10° C. Within two days development was resumed and proceeded normally.

At 2° C. after prolonged periods (four weeks) at the 3-setiger stage many larvae lost their bristles, prototroch, and telotroch and sank to the bottom of the culture vessels, achieving what appeared to be a partial metamorphosis.

The upper temperature limit at which normal growth could take place was not accurately determined. However, temperature tolerance experiments at 30‰ showed that eggs and larvae, like the adults, were able to live for prolonged periods at 30° C. without any serious curtailment of growth. Death occurred between 34° C. and 35° C. The lower limit of temperature at which growth occurred was difficult to determine. Eggs and larvae could be supercooled at least to −5° C. without any harmful effects although none of the specimens appeared able to tolerate the formation of ice crystals in their bodies (*cf.* Kanwisher, 1955). Growth proceeded slowly up to the 3-setiger stage even in specimens held in sea water at −1.5° to −1.7° C.

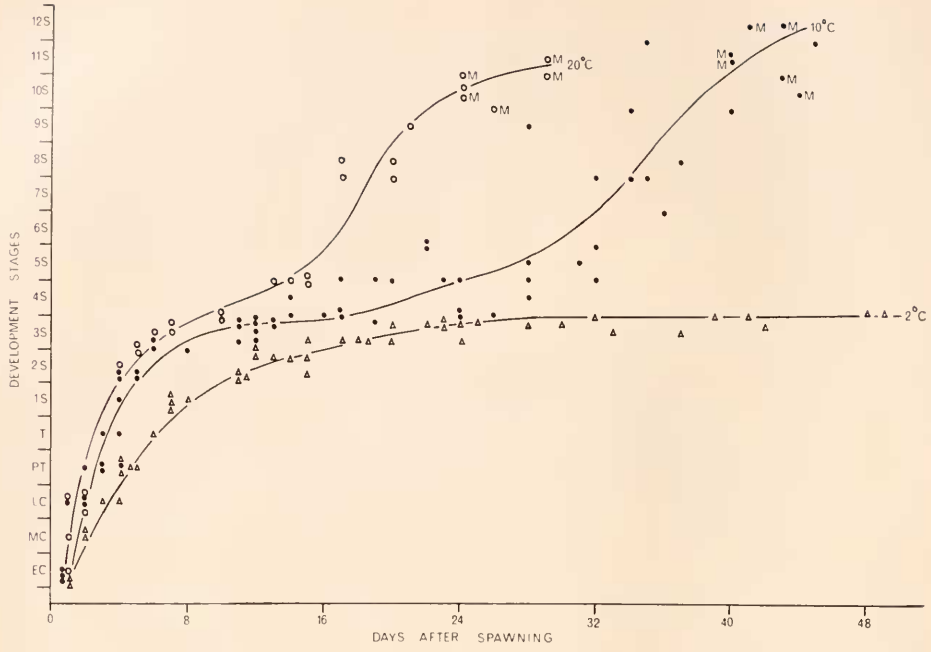


FIGURE 22. The effect of sea-water temperature upon the early development of *S. viridis* at 30‰. M = metamorphosed individuals; EC = early cleavage; MC = mid-cleavage; LC = late cleavage; PT = pre-trochophore; T = trochopore; S = setiger stage. Open triangles = 2° C.; Solid circles = 10° C.; Open circles = 20° C.

Effect of salinity on early development (at 2° C. and 10° C.)

There was no appreciable difference in the rate of development of eggs and larvae in cultures maintained at salinities varying from 10‰ to 30‰ (Table II). At 5‰, however, growth was much slower, development proceeding at only half the rate at 10‰ to 30‰. Below 5‰ growth was interrupted and 2.5‰ proved to be a lethal salinity for eggs and larvae alike. The higher the temperature the more quickly the young stages died at the lower lethal salinities. The reverse was the case at the upper limit of salinity tolerance (45–50‰) where early stages survived longer at higher temperatures (for examples of similar behavior in other animals see Kinne, 1964). All growth ceased in cultures maintained at 45‰.

DISCUSSION

The data presented show clearly that the population of *S. viridis* at Lawrencetown has a short, well-defined, breeding period at the end of March and beginning of April. The observations of Dean and Pettibone of gamete-laden worms in Connecticut and New Hampshire in February, March and early April, conform to this pattern. However, the statement in the unpublished manuscript of Moore that breeding occurs in July and August at Woods Hole is conflicting. Visits to Woods Hole in July of 1963 and 1964 failed to reveal any gamete-bearing speci-

TABLE II

Effect of reduced salinity on the development of young S. viridis at 2° C. and 10° C. Data are from three experiments at each salinity. Numbers denote the setiger stage reached.

2° C.

Days after spawning	5‰	10‰	15‰	20‰	30‰
2	early cleavage	mid-cleavage	late cleavage	late cleavage	late cleavage
8	pre-trochophore	pre-trochophore	trochophore	trochophore	1
14	trochophore	1/2	2	2	2/3
20	1	3	3	3	3
26	2	3	3	3	3
32	2	3	3/4	3	3/4
38	2/3	3/4	3/4	3/4	3/4
44	3	3/4	3/4	3/4	3/4

10° C.

2	mid-cleavage	late cleavage	late cleavage	late cleavage	late cleavage
8	trochophore	1	2	1/4	2/3
14	1	2/3	3	3	3
20	2/3	3/4	4/5	4/5	4/5
26	3	6/7	7	7/8	6/7
32	3/4	8	8	8/9	8
38	4/5	9/10	10	10/11	10
44	5/6	10	10/11	10/11	11

mens of *S. viridis*. It is thus possible that Moore's observations resulted from misidentification.

The onset of gamete production which occurred during October coincided with a fall of temperature after the summer maximum in July, August and September (Fig. 23). It is well known (Orton, 1920; Gunter, 1957; Kinne, 1963) that in many animals a slowly rising or falling temperature induces gonadal development, and this may well be the stimulus for gamete production in the Lawrencetown population. According to Gunter (1957) it is usual that where temperature change is acting as a stimulus to spawning, spawning is induced by the same direction of temperature change that leads to gonadal development. In the case of *S. viridis*, however, it would appear that if temperature change is acting at all as an inducement to spawning, then it is a temperature rise in the spring that is the effecting agent. Although gamete production starts whilst temperatures are falling and continues during the coldest months of the year, it is quite possible that a rise in temperature is needed to complete the final ripening of the gametes and to bring on spawning. Indeed, if apparently mature worms are kept at a constant low temperature of approximately 0° C. they can be held for many months without spawning and will eventually reabsorb their sexual products (*cf. Crassostrea virginica*—Loosanoff and Davis, 1951). Conversely, by slowly raising the temperature from 0° C. to 2° C. over a two-week period worms can be made to spawn under laboratory conditions, up to a month before natural spawning in the field.

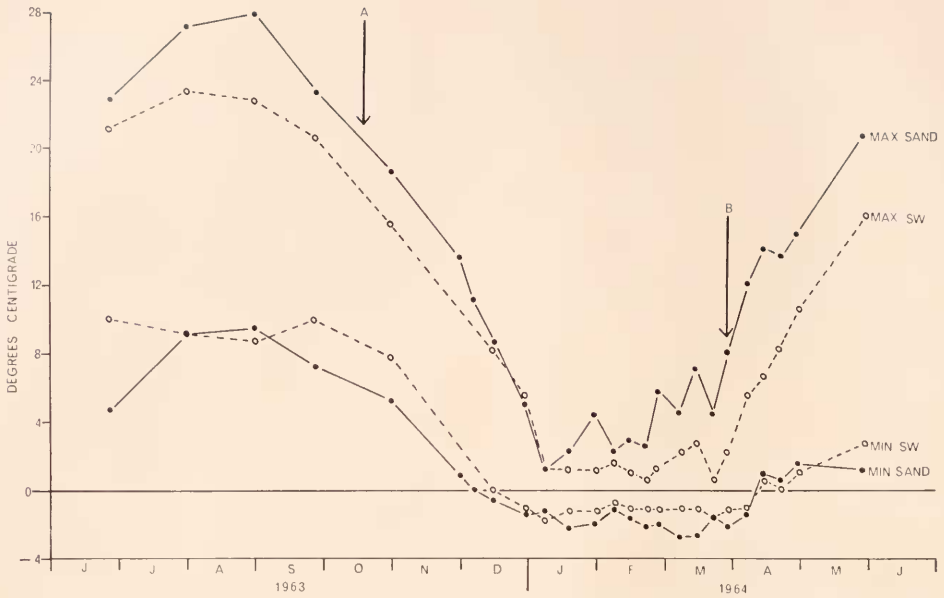


FIGURE 23. Maximum-minimum temperatures for the sea water overlying the sampling site (open circles, broken line) and for the mud-sand 1 cm. below the surface (solid circles, unbroken line). Arrows mark (A) the onset of gamete production, and (B) the time of spawning.

Sudden temperature changes seemed ineffective as a spawning stimulus (*cf. Cirriformia tentaculata*—George, 1964). On the other hand sudden changes of salinity such as occur over a tidal cycle at the sampling site induced some spawning in ripe worms.

Since spawning can be prevented by keeping the temperature at a constant low level it is unlikely that spawning occurs as a result of a biological clock mechanism or lunar periodicity.

The fact that eggs are not fertilizable nor cleavage possible below 5‰ is of interest, since 3-setiger larvae are able to live at a salinity of 2.5‰ (although not grow), and adults can tolerate a salinity of 0.5‰ for an indefinite period. A similar phenomenon has recently been reported for *Nereis diversicolor* by Smith (1964), although in these worms the range of tolerance for cleavage and successful gastrulation is narrower than for fertilization. It is likely that the susceptibility of early development stages of the worm to low salinities limits the spread of reproductively viable individuals into the upper reaches of estuaries. At Lawrencetown adults were found living permanently in areas where the salinity never rises to 5‰. These worms were presumably either passively transported there as larvae or had reached there by active swimming in the adult phase (it has already been noted that the adults are good swimmers). It is unlikely that these worms migrate back to areas of higher salinity during the breeding period, and they are probably non-reproductive individuals.

A burst of phytoplankton occurred during April of both 1963 and 1964 and

continued on until the end of May. An abundance of phytoplankton at this time is extremely valuable to the larvae of *S. viridis,* ensuring them adequate nourishment when their yolk supply is exhausted. Some of the phytoplankton was of a size suitable for direct ingestion by the larvae but the increase in readily-ingestible organic detritus as a result of breakdown of the phytoplankton was probably of greater importance to the larvae.

Water temperature is also important to the Lawrencetown population, for the temperature must rise above 2° C. before the larvae have reached the 3-setiger stage or else development is suspended. Possibly this minimal temperature requirement limits the northern spread of the worm to Labrador and into the Arctic Circle, for the lower temperature tolerance levels of larvae and adults are not limiting as such. The southern limit of distribution of the worm can similarly be explained in terms of temperature. According to the surface sea-water temperature data for 1953–54 accumulated by Pyle (1962) the average August temperature in Georgia and most of the east coast of Florida exceeded 30° C., a temperature that is near the upper limit for growth of *S. viridis.* If these data are typical for these regions, then temperatures of several degrees higher can be expected as a matter of course close to shore and in estuaries. These temperatures may well exceed the upper limit of temperature tolerance (34° C.) for the species.

If spawning of *S. viridis* takes place in March and April over its whole range of distribution (as is indicated by accumulated data), then the length of time spent by the worms in the plankton will be much less towards the worms' southern limit. Using the temperature/growth curves constructed from the laboratory experiments as a guide to the variation of the growth rate with temperature, one can deduce that metamorphosis is reached in approximately four weeks in South Carolina compared with almost two months in Nova Scotia.

The author is indebted to Dr. Marian Pettibone and Dr. David Dean for supplying information and to Dr. Eve Southward for critically reading the manuscript.

Summary

1. A redescription of the external morphology of *Scolecolepides viridis* (Verrill) is given.

2. From data obtained by regular sampling of a population at Lawrencetown, Nova Scotia, it was found that the worm has a short breeding period in late March and early April.

3. The onset of gamete formation coincides with a fall in the temperature following the summer maximum. Gametes occur in the coelom along the greater part of the animal's length and are shed through mixonephridia. Spawning is due to rising temperatures in the spring. In addition some spawning may result from sudden changes in salinity during a tidal cycle.

4. The thick-walled eggs containing membrane vesicles and the short-headed sperm are probably shed whilst the worms are still in their burrows. Larval development is entirely planktonic.

5. In laboratory cultures maintained at 10° C. and 30‰ photopositive trochophores begin active swimming in 3–4 days, and the 3-setiger stage is reached in 10

days. The larvae start to metamorphose at the 9- to 10-setiger stage, but metamorphosis can be postponed until the 13-setiger stage if the substratum is not suitable. The fully-metamorphosed 10-setiger worm is 750 μ long and 40 days old.

6. The rate at which development proceeds in laboratory cultures is dependent on temperature and salinity. At 20° C. metamorphosis is reached in approximately half the time taken at 10° C. At 2° C. no larvae develop beyond the 3- to 4-setiger stage. The rate of development is similar in salinities varying from 10‰ to 30‰. At 5‰, however, growth ceases.

7. An attempt is made to relate the distribution of the worm along the east coast of North America to temperature.

LITERATURE CITED

Cowles, R. P., 1931. A biological study of the offshore waters of Chesapeake Bay. *Bull. Bur. Fish., Wash.*, **46**: 277–381.

Dean, D., 1965. On the reproduction and larval development of *Streblospio benedicti* Webster. *Biol. Bull.*, **128**: 67–76.

Franzén, A., 1956. On spermiogenesis, morphology of the spermatozoon, and biology of fertilization among invertebrates. *Zool. Bidr. Upps.*, **31**: 355–482.

George, J. D., 1964. The life history of the cirratulid worm, *Cirriformia tentaculata*, on an intertidal mudflat. *J. Mar. Biol. Assoc.*, **44**: 47–65.

Goodrich, E. S., 1945. The study of nephridia and genital ducts since 1895. *Quart J. Micr. Sci.*, **86**: 113–392.

Gunter, G., 1957. Temperature. *In:* Treatise on Marine Ecology and Paleoecology, vol. 1. *Mem. Geol. Soc. Amer., No. 67: 159–184.*

Hannerz, L., 1956. Larval development of the polychaete families Spionidae Sars, Disomidae Mesnil, and Poecilochaetidae N. Fam. in the Gullmar Fjord (Sweden). *Zool. Bidr. Upps.*, **31**: 1–204.

Joyner, A., 1962. Reproduction and larval life of *Nerine cirratulus* (Delle Chiaje) family Spionidae. *Proc. Zool. Soc. London*, **138**: 655–666.

Kanwisher, J. W., 1955. Freezing in intertidal animals. *Biol. Bull.*, **109**: 56–63.

Kinne, O., 1963. The effects of temperature and salinity on marine and brackish water animals. I. Temperature. *Oceanogr. Mar. Biol. Ann. Rev.*, **1**: 301–340.

Kinne, O., 1964. The effects of temperature and salinity on marine and brackish water animals. II. Salinity and temperature salinity combinations. *Oceanogr. Mar. Biol. Ann. Rev.*, **2**: 281–339.

Loosanoff, V. L., and H. C. Davis, 1951. Repeated semiannual spawning of northern oysters. *Science*, **115**: 675–676.

Mead, A. D., 1897. The early development of marine annelids. *J. Morph.*, **13**: 227–236.

Orton, J. H., 1920. Sea temperature, breeding and distribution of marine animals. *J. Mar. Biol. Assoc.*, **12**: 339–366.

Pyle, R. L., 1962. Sea surface temperature regime in the Western North Atlantic 1953–1954. Folio I of: Serial Atlas of the Marine Environment. American Geographical Society, New York.

Sanders, H. L., E. M. Goudsmit, E. L. Mills and G. E. Hampson, 1962. A study of the intertidal fauna of Barnstable Harbor, Massachusetts. *Limnol. Oceanogr.*, **7**: 63–79.

Smith, R., 1964. Keys to marine invertebrates of the Woods Hole region. No. 11. Systematics-Ecology Program, Marine Biological Laboratory, Woods Hole, Massachusetts.

Smith, R., 1964. On the early development of *Nereis diversicolor* in different salinities. *J. Morph.*, **114**: 437–464.

Stickney, A. P., 1959. Ecology of the Sheepscot River estuary. *Spec. Sci. Rep. U. S. Fish Wildl. Serv.*, **309**: 1–21.

Thorson, G., 1950. Reproduction and larval ecology of marine bottom invertebrates. *Biol. Rev.*, **25**: 1–45.

WEBSTER, H. E., 1879. Annelida Chaetopoda of New Jersey. *Rep. N. Y. State Mus. Nat. Hist.*, **32**: 101–128.

WEBSTER, H. E., AND J. E. BENEDICT, 1884. The Annelida Chaetopoda from Provincetown and Wellfleet, Mass. *Rep. U. S. Comm. Fish., vol. for 1881: 699–747.*

WELLS, H. W., AND I. E. GRAY, 1964. Polychaetous annelids of the Cape Hatteras area. *J. Elisha Mitchell Sci. Soc.*, **67**: 133–161.

WILSON, D. P., 1928. The larvae of *Polydora ciliata* Johnston and *Polydora hoplura* Claparède. *J. Mar. Biol. Assoc.* **15**: 567–603.

VERRILL, A. E., 1873. Report upon the invertebrate animals of Vineyard Sound and the adjacent waters, with an account of the physical characters of the region. *Rep. U. S. Comm. Fish., vol. for 1871–1872: 295–778.*